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PALM INTRANET

Continuity Information for 10/726134

Parent Data
10726134
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Child Data
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Appln Info | Contents | Petition Info | Atty/Agent Info | **Continuity Data** | Foreign Data | Invent

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TABLE 1

Sample	Antibody (LPS)	*E. coli*	*S. aureus*	PBS	Liquid Crystal	Bire-fringence
A	500 ng	4.6×10^1	—	—	5 μl	YES
B	500 ng	—	—	5 μl	5 μl	NO
C	500 ng	—	2×10^6	—	5 μl	NO

In other experiments, LPS antibody, *E. Coli* (2.7×10^7 CFU) and liquid crystalline material were reacted in a manner similar to the previous experiment, and representative photomicrographs (110× magnification; FIGS. **4A**, **4B** and **4C**) were evaluated with a Bio-Quant Image Analysis System. The image analysis was performed to quantatively compare propagating light transmission when *E. coli*, PBS or *S. aureus* was evaluated by the receptor-ligand binding system. The photographic images were digitized and integrated optical density (IOD) automatically calculated according to the following formula:

$$IOD = \frac{(\sum -\log_{10})(\text{foreground})}{(\text{background})}$$

The resulting data, presented in FIG. **5**, show that a profound increase in transmission of propagating light occurs when liquid crystals amplify the binding of antibody to *E. coli* (LPS).

Caged Enzyme Amplification Mechanism

The sensitivity of an alpha-2-macroglobulin amplification mechanism was demonstrated. Standard 2 mg/100 μl solution of human alpha-2-macroglobulin (α_2M, Calbiochem Co., product number 441251), trypsin (Sigma Chemical Co., T-8003) and thrombin (Sigma Chemical Co., T-4648) were made by dissolving the protease or antiprotease in 0.1 M HEPES buffer (pH 7.6). Equal volumes (100 μl) of α_2M and one of the enzymes were mixed, permitted to interact for ten minutes at room temperature and then cooled to 4° C. The 200 μl sample was added to a gel filtration column (1 cm×24 cm; 18.8 ml bed volume; 0.44 ml/min flow rate; located in a 4° C. cold room) packed with Sephadex G-100 to separate the caged enzyme from uncomplexed enzymes. Column eluent was collected in 1.0 ml fractions. Changes in light absorbance at 280 nm was measured to determine the protein concentration in each fraction and enzymatic activity was determined to identify those fractions containing the caged enzymes. The results of those measurements, shown in FIGS. **6A** and **6B**, demonstrate that fractions 15–17 contained relatively pure samples of caged enzyme. Those fractions were used for the subsequent evaluations.

Small synthetic substrates, N-benzoyl-L-arg-p-nitroanalide and N-p-tosyl-gly-pro-arg-p-nitroanalide, were used to define the enzymatic activities of caged trypsin (FIG. **6C**) and caged thrombin (FIG, **6D**), respectively. While both systems exhibited dose-response characteristics, the caged thrombin exhibited greater sensitivity. The enzymatic activity did not degrade with time. The enzymatic activity of the caged trypsin was unchanged following six days of storage at 4° C. (FIG. **6E**). Similarly, caged thrombin activity was also stable when measured 24 hours following preparation.

Luciferase Amplification Mechanism

The exceptional sensitivity of a luciferase-based amplification mechanism was demonstrated using a Berthold Lumat Luminometer. Varying amounts of luciferase (4 mg/ml of 0.15% NaCl, 10 mM HEPES, 1 mM EDTA, 2 mM $MgCl_2$, 2 mM dithiothreitol; Sigma Chemical Co.) were added to the luminometer reaction chamber. The enzymatic reaction was initiated by rapid injection of 0.5 mM luciferin (Promega, E1483), 0.5 mM adenosine triphosphate (Sigma Chemical Co., A-7699), 5 mM $MgSO_4$, 1.0 mM dithiothreitol (Sigma Chemical Co.) in 50 mM HEPES buffer (pH 7.8) into the reaction chamber. FIG. **7B** shows that detectable luciferase activity can be measured with an enzyme concentration of only 0.0017 pg/μl, and linear increases in activity are observed with progressive elevations in enzyme concentration.

It is to be understood that any variations evident fall within the scope of the claimed invention, and thus the selection of specific antibodies, caged enzymes, receptor-inactivated enzymes or liquid crystals can be determined without departing form the spirit of the invention herein disclosed and described. It should also be understood that the present invention, while particularly suited for pathogen detection, is intended to include the detection of any ligand. Moreover, the scope of the invention shall include all modifications and variations that may fall within the scope of the attached claims.

We claim:

1. A device for the detection of ligands comprising:

at least one receptor capable of binding to a ligand to form a receptor-ligand complex, wherein the formation of the receptor-ligand complex produces a signal; and

an amplification mechanism, wherein said amplification mechanism is a lyotropic liquid crystalline material coupled to the receptor, and wherein said amplification mechanism amplifies said signal upon receptor-ligand complex formation.

2. The device of claim **1**, wherein the receptor is an antibody selected from the group consisting of monoclonal, polyclonal and molecularly engineered antibodies, wherein said antibodies form a signal-producing receptor-ligand complex when the receptor binds to the ligand.

3. The device of claim **1**, wherein the ligand is a pathogenic agent.

4. The device of claim **1**, wherein the amplified signal is generated by a change in optical characteristics of the lyotropic liquid crystalline material.

5. A device for the detection and monitoring of the presence of ligands comprising:

multiple wells, each well having a predetermined receptor therein, wherein said receptor is capable of binding to a ligand, and wherein the formation of the receptor-ligand complex produces a signal; and

an amplification mechanism, wherein said amplification mechanism is a lyotropic liquid crystalline material coupled to the predetermined receptor, and wherein said amplification mechanism amplifies said signal upon binding of a specific ligand to its predetermined receptor.

6. The device of claim **5**, wherein the receptor is an antibody, wherein said antibody forms a signal-producing receptor-ligand complex upon ligand binding.

7. The device of claim **6**, wherein the antibody is selected from the group consisting of monoclonal, polyclonal and molecularly engineered antibodies.

8. The device of claim **5**, wherein the amplified signal is transduced into an optically perceptible signal.

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